15. (Amended) The method of claim 10, wherein said transgenic plant is tobacco and has enhanced resistance to tobacco mosaic virus.

16. (Amended) The method of claim 10, wherein said transgenic tobacco plant has enhanced resistance to species of the fungal genus *Phytophthora*.

Please cancel Claims 2, 3, 6, 8, 9, 11, 14, 16, and 17.

A marked-up copy of the amendments presented herein is provided in Appendix A.

REMARKS

The January 15, 2002 Official Action and references cited therein have been carefully reviewed. In light of the amendments presented herewith and the following remarks, favorable reconsideration and allowance of the application are respectfully requested.

At the outset, the Examiner has indicated that there are certain minor informalities in the application which require correction. Specifically, a priority claim and sequence identifiers have been inserted into the specification where appropriate. The Brief Descriptions of the Drawings have been amended to more accurately describe those figures having multiple parts. Additionally, the minor grammatical errors in claims 14-17 have been rectified. An amended Declaration has been forwarded to the inventors for execution. This Declaration will be forwarded to the United States Patent Office immediately upon receipt in the offices of the undersigned.

At page 3 of the Official Action, the Examiner has rejected Claims 1-17 under 35 U.S.C. §112, second paragraph, as allegedly indefinite for failing to particularly point out and distinctly claim the subject matter regarded as the

invention.

The Examiner has also rejected Claims 1-17 under 35 U.S.C. §112, first paragraph, as allegedly containing subject matter not described in the specification in such a way as to reasonably convey to one of skill in the art that the inventors had possession of the claimed invention at the time the application was filed.

At page 7 of the Official Action, the Examiner has further rejected Claims 1-17 under 35 U.S.C. §112, first paragraph, asserting that the specification allegedly fails to provide enablement commensurate in scope with the present claims.

At page 11 of the Official Action, the Examiner has rejected Claims 1-3, 6-11 and 13-17 under 35 U.S.C. §102(b) as allegedly anticipated by Seo et al. (Science, 1995). In addition, the Examiner has rejected Claims 1-17 under 35 U.S.C. §103(a) as allegedly unpatentable over Seo et al. in view of Gatz et al. (Mol. Gen. Genet., 1991).

The foregoing constitutes the entirety of the objections and rejections raised in the January 15, 2002 Official Action. In light of the present claim amendments and the following remarks, each of the above-noted rejections under 35 U.S.C. §§ 112, first and second paragraphs, 102 and 103 is respectfully traversed. Applicants respectfully submit that the claims are in condition for allowance. Accordingly, early and favorable action on this application is earnestly solicited.

CLAIMS 1-17 AS AMENDED FULLY COMPLY WITH THE DEFINITENESS REQUIREMENTS OF 35 U.S.C. §112, SECOND PARAGRAPH

The Examiner has rejected Claims 1-17 under 35 U.S.C. §112, second paragraph, as allegedly indefinite for failing to particularly point out and distinctly claim the subject matter regarded as the invention.

The relevant inquiry in determining whether a given

claim satisfies the requirements of 35 U.S.C. §112, second paragraph, is whether the claim sets out and circumscribes a particular area with a reasonable degree of precision and particularity such that the metes and bounds of the claimed invention are reasonably clear. In re Moore, 169 U.S.P.Q. 236 (C.C.P.A. 1971). Applicants respectfully submit that with respect to Claims 1-17 as amended, this inquiry must be answered in the affirmative.

The Examiner asserts that the name "WIPK" does not clearly identify the enzyme or coding sequence presently claimed. In addition, the Examiner contends that in view of the teachings of the present application and by Zhang and Klessig (PNAS, 1998), it is not clear that homologs of the cDNA taught by Seo et al. (Science, 1995) would be designated "WIPK" or that WIPK orthologs would be designated wounding-activated kinases.

Given this apparent confusion, the claims have been amended to more accurately describe the present invention. Specifically, Claim 1 has been amended to recite a transgenic plant that is produced using a DNA construct containing a cDNA molecule encoding WIPK enzyme or an enzyme that has 90% sequence identity thereto which was originally disclosed by Seo et al. The Seo et al. manuscript is referred to throughout the present specification has been incorporated by reference therein. See page 1, lines 20-22. As indicated in Seo et al., this cDNA sequence was submitted to GenBank and was assigned Accession No. D61377. Inasmuch as Seo et al. was incorporated by reference, Applicants submit that this amendment does not introduce new matter into the claims. Furthermore, it is a well-settled premise in patent law that a patent need not teach, and preferably omits, what is well known in the art. Lindemann Maschinenfabrik v. American Hoist and Derrick, 221 USPQ 481, 489 (Fed. Cir. 1984).

Support for an enzyme having 90% identity to the WIPK enzyme encoded by the nucleic acid of GenBank accession

number D61377 can be found at page 16, lines 10-13. Applicants assert that this amendment serves to specifically identify the cDNA molecule encoding the WIPK enzyme of Applicants claims for the generation of transgenic plants that have enhanced resistance to pathogens. Claim 10 has been amended in a similar manner. It is Applicants' position that these claim amendments serve to remove any perceived lack of clarity from the claims.

Claims 1, 11 and 12 have also been amended to provide proper antecedent basis for the claimed subject matter. Specifically, "said cell" in Claim 1 has been replaced with "plant cells". Claims 11 and 12 have been amended to replace the phrase, "WIPK protein" with "a WIPK enzyme".

In view of the claim amendments presented herewith, Applicants respectfully submit that one of skill in the art would be readily apprised of the metes and bounds of the claims. Accordingly, the rejection of Claims 1, 2, 4, 6 and 10-13 as amended under 35 U.S.C. §112, second paragraph, is no longer appropriate and should be withdrawn.

CLAIMS 1-17 AS AMENDED ARE FULLY DESCRIBED AND ENABLED BY THE DISCLOSURE IN THE SPECIFICATION

The Examiner has rejected Claims 1-17 under 35 U.S.C. §112, first paragraph, as allegedly containing subject matter which was not described in the specification in such a way as to reasonably convey to one of ordinary skill in the art that, at the time the application was filed, the inventors had possession of the claimed invention. The Examiner also contends that the specification is not commensurate in scope with the claimed subject matter.

A rejection under 35 U.S.C. §112, first paragraph based on inadequate enablement is proper only when the rejected claims are of such breadth as to read on subject matter as to which the specification is not enabling. In re

Borkowski 164 U.S.P.Q. 642 (CCPA 1970).

Applicants respectfully submit that Claims 1-17 as amended are fully described and enabled by present specification. Claim 1 has been amended to call for transgenic plant having enhanced resistance to agents selected from the group consisting of tobamoviruses, elicitin-producing, parasiticein-producing, cyrptogein-producing fungi, harpin-producing bacteria, tobacco mosaic virus and Phytophthora fungi. Support for this amendment can be found at page 15, lines 10-14, and at page 5, line 1. Thus, the Examiner's assertion that the specification fails to provide any guidance regarding other stimulators of WIPK activation is factually erroneous.

As discussed in connection with the rejection of claim 1 under 35 U.S.C. §112, second paragraph, claim 1 has been further amended to call for a DNA construct comprising the nucleic acid sequence set forth in Genbank Accession No. D61377 or a sequence which is 90% identical thereto encoding an enzyme having WIPK function. The claim has also been amended to recite that the transgenic plants express the N gene.

In light of the foregoing claim amendments and remarks, Applicants respectfully submit that the claims are amended comply with all the requirements of 35 U.S.C. §112, first paragraph and request that the rejection of amended claims 1-17 under 35 U.S.C. §112, first paragraph be withdrawn.

CLAIMS 1-3, 6-11 AND 13-17 AS AMENDED ARE NOT ANTICIPATED BY SEO ET AL.

The Examiner has rejected Claims 1-3, 6-11 and 13-17 under 35 U.S.C. §102(b) as allegedly anticipated by Seo et al.

A rejection under 35 U.S.C. §102(b) is warranted only when the cited reference identically discloses the

subject matter of the invention as claimed. In re Bond, 15 U.S.P.Q. 2d 1566 (Fed. Cir. 1990). Applicants respectfully submit that Seo et al. do not identically disclose each and every element of the claimed invention.

Claims 1 and 10 have been amended to call transgenic plants and a method for producing the same by transforming plants with a DNA construct containing the figwort mosaic virus 35S promoter operably linked to the nucleic acid sequence encoding the tobacco WIPK enzyme (GenBank Accession NO. D61377) or a sequence 90% identical thereto encoding a functional WIPK enzyme. The construct disclosed by Seo et al. contained the 35S Cauliflower mosaic virus promoter as opposed to applicants constructs which contain the figwort mosaic 35S promoter. Accordingly the transgenic plants and methods of applicants claims are not identically disclosed by Seo et al. Based on the foregoing remarks and claim amendments, Applicants respectfully submit that the disclosure of Seo et al. fails to anticipate the claimed invention, and request that the rejection of Claims 1-3, 6-11 and 13-17 under 35 U.S.C. §102(b) be withdrawn.

CLAIMS 1-17 AS AMENDED ARE NOT RENDERED OBVIOUS BY SEO ET AL. IN VIEW OF GATZ ET AL.

At the outset, Applicants note that the Examiner is correct in his assumption that the subject matter of the present invention is commonly owned.

The Examiner has rejected Claims 1-17 under 35 U.S.C. §103(a) as allegedly unpatentable over Seo et al. in view of Gatz et al. (Mol. Gen. Genet., 1991). It is the Examiner's position that it would be obvious to one of ordinary skill in the art to modfify the plants of Seo et al. using the tetracycline repressor/operator controlled promoter taught by Gatz et al.

The relevant inquiry in determining obviousness under 35 U.S.C. §103 based on the combined disclosure of

references, is whether the references supply some teaching or suggestion to one of ordinary skill in the art to arrive at the invention as claimed. In re Dow Chemical Company, 5 U.S.P.Q.2d 1529 (Fed. Cir. 1988). Obviousness cannot be established by combining the teachings of the prior art to produce the claimed invention, absent some teaching or suggestion supporting the combination. In re Fine, 5 U.S.P.Q.2d 1596 (Fed. Cir. 1988). Moreover, the teaching or suggestion supporting the desirability or the combination must be found in the prior art, not in the applicant's disclosure. In re Fritch, 23 U.S.P.Q.2d 1780 (Fed. Cir. 1992). Under these standards, none of the cited references, considered singly or in combination, renders obvious the claimed invention.

Nowhere in the disclosure of Seo et al. is there any motivation, teaching or suggestion to modify the promoter utilized to express the WIPK encoding DNA construct for the creation of disease resistant transgenic plants. Indeed, Gatz et al. was published four years prior to the Seo et al. manuscript. If modifications to the construct were obvious, as asserted by the Examiner, the means to do so were at the disposal of Seo et al. who **chose** not to create DNA constructs under the control of an inducible promoter. Rather, Seo et al. used a constitutive promoter in order to generate transgenic plants for testing the function of the WIPK protein.

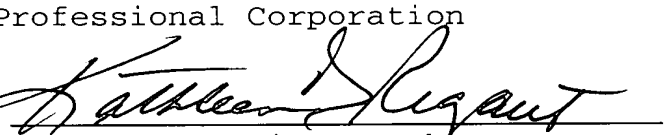
After reviewing each of the prior art references cited by the Examiner, Applicants strenuously assert that the §103 rejection of Claims 1-17 in this application is a classic case of hindsight reconstruction of the claimed invention. It is quite apparent that the Examiner has used Applicants' disclosure as a guide for combining unrelated prior art disclosures in an effort to make out a case of obviousness. Applicants are unaware of any other way the Examiner could possibly have combined these references to arrive at the claimed invention without using Applicants' disclosure as a

template especially since these prior art references fail to suggest when an inducible promoter may be preferred for use when creating transgenic plants that exhibit enhanced disease resistance. Accordingly, Applicants respectfully submit that the rejection of Claims 1-17 under 35 U.S.C. §103(a) is untenable and must be withdrawn.

In view of the amendments and remarks presented herein, it is respectfully urged that the rejections set forth in the January 15, 2002 Official Action be withdrawn and that this application be passed to issue. In the event the Examiner is not persuaded as to the allowability of any claim, and it appears that any outstanding issues may be resolved through a telephone interview, the Examiner is requested to telephone the undersigned attorney at the phone number given below.

Respectfully submitted,

DANN, DORFMAN, HERRELL AND SKILLMAN
A Professional Corporation

By 
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Enclosures: Appendix A

Appendix A

In the Specification:

Please insert at Page 1, Line 1 the following statement:

This application is a §371 application of PCT/US99/03882, filed February 23, 1999 which in turn claims priority to US Provisional Application No. 60/075,685, filed February 24, 1998.

(Page 5, Line 29) **[Figure 1] Figures 1A and 1B.** Activation of 48-kD and 44-kD kinases in TMV-infected tobacco. Tobacco plants carrying *N* resistance gene (*N. tabacum* cv Xanthi nc [NN]) were inoculated with either TMV (U1 strain, 1 μg/mL in 50 mM phosphate buffer, pH 7.0) or buffer only (mock). After infection, plants were maintained at 32°C for 48 hr. Discs from the infected leaves were collected at various time after the plants were shifted back to 22°C (hps, hr post shift) and protein extracts were prepared. **Fig. 1A:** In-gel kinase activity assay. Extracts containing 15 μg protein were electrophoresed in 10% SDS-polyacrylamide gels imbedded with 0.25 mg/mL of MBP in the separating gel. After protein renaturation, the kinase reaction was carried out as described in Materials and Methods. The sizes of activated kinases are given in kilodaltons. **Fig. 1B:** The activities of 48-kD kinase (in TMV- [●] and mock- [○] inoculated leaves) and 44-kD kinase (in TMV- [▲] and mock- [△] inoculated leaves) were quantitated using a PhosphorImager and the relative activities were plotted against time. Kinase activities were normalized to the level present at the zero time point for the 48-kD kinase, which was given a value of 1.

(Page 6, Line 17) **[Figure 2] Figures 2A-2C.** Immuno-complex kinase assays using sequence-specific antibodies against SIPK and WIPK. **Fig. 2A:** An antibody

raised against a peptide (p44N) corresponding to the unique N-terminus of WIPK, Ab-p44N, specifically recognized the WIPK protein. Two nanograms each of recombinant HisSIPK, HisNtf4, HisWIPK, and HisNtMPK6 or 20 μg of protein extracts from 48 hr mock- or TMV-inoculated tobacco leaves (maintained throughout infection at 22°C) were subjected to immunoblot analysis with Ab-p44N in the absence or presence of 0.2 μg/mL competitor peptides p44N or p48N. **Fig. 2B:** Immuno- complex kinase assay of TMV-activated kinase using SIPK-specific antibody, Ab-p48N. Protein extracts (50 μg) from TMV- or mock-inoculated leaf tissue were reacted with Ab-p48N (2.5 μg). The resultant antigen-antibody complex were precipitated with protein A-agarose beads, washed extensively before addition to a kinase assay mixture with [γ-^{32}P]-ATP and MBP as substrates. The reaction mixture, including the phosphorylated MBP, were then fractionated by SDS-PAGE. **Fig. 2C:** Immuno-complex kinase assay of TMV-activated kinase using WIPK-specific antibody, Ab-p44N. Protein extracts (50 μg) from TMV- or mock-inoculated leaves were immunoprecipitated with Ab-p44N (2.5 μg) and the kinase activity of the immuno-complex was determined as above. Times in B and C are given in hps from 32°C to 22°C.

(Page 7, Line 9) **[Figure 3]** **<u>Figures 3A and 3B</u>.** Activation of *WIPK* gene expression by TMV in tobacco plants (cv Xanthi nc [NN]) after temperature shift. **Fig. 3A:** Increase in steady-state levels of *WIPK* mRNA in TMV-infected plants. Duplicates of leaf discs used in Figure 1 were extracted for total RNA, thus facilitating direct comparison of the induction kinetics of mRNA and enzymatic activity. Twenty micrograms of total RNA per lane were separated on 1.2% formaldehyde-agarose gels and transferred to Zeta-probe membranes. Blots were hybridized with random primer-labeled inserts consisting of either a full-length cDNA of *WIPK* (data shown) or its 3'-untranslated region (data not shown). **Fig.**

3B: Increase of WIPK protein in TMV-infected tobacco after temperature shift. Samples containing 20 μg of protein from the leaf extracts used for Fig. 1A were separated on 10% SDS-polyacrylamide gels. After blotting to nitrocellulose, the WIPK protein was detected with Ab-p44N.

(Page 7, Line 27) **[Figure 4] Figures 4A-4C.** Activation of WIPK by TMV in tobacco plants (cv Xanthi nc [NN]) maintained at 22°C throughout infection. **Fig. 4A:** Increase in steady-state levels of *WIPK* mRNA in TMV-infected tobacco plants. Tobacco plants were inoculated with TMV or buffer (mock) as in Figure 1 except a higher concentration of TMV was used (5 μg/mL). Leaf discs were taken at the indicated times in hr post inoculation (hpi). Total RNA was prepared and analyzed for *WIPK* mRNA as described in Figure 3. **Fig. 4B:** Increase of WIPK protein in TMV-infected tobacco maintained at 22°C. Protein extracts were prepared from duplicate leaf discs to those used in Fig. 4A. Twenty micrograms of protein was analyzed by immunoblotting using Ab-p44N as described in Figure 3. **Fig. 4C:** Induction of WIPK enzymatic activity in TMV-infected tobacco maintained at 22°C. Selected protein extracts from (B) were analyzed by immuno-complex kinase assay using WIPK-specific Ab-p44N as described in Figure 2.

(Page 8, Line 26) **[Figure 6] Figures 6A-6C.** TMV activation of *WIPK* transcription in tobacco is *N* gene dependent, SA independent and systemic. **Fig. 6A:** *WIPK* mRNA induction in tobacco by TMV infection is *N* gene dependent. TMV-susceptible tobacco plants (*N. tabacum* cv Xanthi [nn] which lacks *N* resistance gene) were infected and *WIPK* mRNA detected by RNA gel blot analysis. **Fig. 6B:** Induction of *WIPK* mRNA by TMV infection is SA independent. Transgenic tobacco (cv Xanthi nc [NN]) plants expressing the *NahG* gene were infected and *WIPK* mRNA determined by RNA gel blot

analysis. **Fig. 6C:** Systemic induction of *WIPK* mRNA after TMV infection. Three leaves from each tobacco plants (cv Xanthi nc [NN]) were either inoculated with TMV or buffer only (mock) and maintained at 22°C. At indicated days post inoculation (dpi), leaf discs were taken from the upper uninoculated leaves. Total RNA was isolated and *WIPK* mRNA levels were determined.

(Page 9, Line 9) **[Figure 7] Figures 7A and 7B.** Autoradiograms of immunoblot assays showing that the 48-kD MBP kinase activated by water infiltration and wounding is encoded by *SIPK* rather than *WIPK*. Protein extracts (50 µg) from water-infiltrated, cutting or abrasion-wounded leaves were immunoprecipitated with either the SIPK-specific antibody Ab-p48N **(Fig. 7A)** or the WIPK-specific antibody Ab-p44N **(Fig. 7B)**. Kinase activity of the resultant immuno-complexes was subsequently determined as described in Example 1.

(Page 9, Line 19) **[Figure 8] Figures 8A and 8B.** Autoradiograms of RNA or immunoblot assays showing that water infiltration and wounding induce transient increases in *WIPK* mRNA levels, but little or no increases in WIPK protein level. **Fig. 8A:** Total RNA was extracted at the indicated times from water infiltrated or wounded leaves and subjected to RNA gel blot analysis. Blots were sequentially hybridized with the 3' UTR and then the full length *WIPK* cDNA. Both probes yielded the same result; thus, only the autoradiogram produced with the full-length cDNA is shown. **Fig. 8B:** Protein extracts (20µg) were subjected to immunoblot analysis with the WIPK-specific antibody, Ab-p44N.

(Page 26, Line 18) Once cloned, a constitutively activated WIPK kinase may be constructed, as has been done for

mammalian (Mansour et al., 1994) and *Xenopus* (Gotoh et al., 1994) MAPK kinases. MAPK kinases are activated by dual phosphorylation of a SXXXS/T motif (SEQ ID NO: 1) in the kinase subdomain VIII by MAPK kinase kinase. Substitution of these two Ser/Thr residues with Asp or Glu was found to increase basal activity about 100 fold, and cells transformed with these mutants exhibited constitutive activation of the MAPK regulated pathway (i.e. AP-1 transcription; Mansour et al., 1994). The constitutively activated mutatn WIPK will be transformed into plant cells and the corresponding transgenic plants obtained. These plants will have the WIPK-mediated signal transduction pathway constitutively activated.

(Page 28, line 29) **Antibody production and immunoblot analysis.** The peptides p44N (MADANMGAGGGQFPDFPS; SEQ ID NO: 2) and p48N (MDGSGQQTDTMMSDAGAEQPPTAP; SEQ ID NO: 3), which correspond respectively to the unique N-termini of the WIPK and the SIPK proteins, were synthesized and conjugated to keyhole limpet hemacyanin (KLH) carrier. Polyclonal antisera were raised in rabbits and purified by affinity column chromatography (Zymed Laboratory, South San Francisco, CA).

(Page 31, Line 34) **The 44-kD kinase activated by TMV is encoded by *WIPK*.** The size and substrate preference of the 44-kD kinase suggested that it also might be a MAP kinase, possibly that encoded by *WIPK*. To confirm or refute this possibility, antibody was prepared in rabbits against a peptide corresponding to the unique N-terminus (p44N, MADANMGAGGGQFPDFPS; SEQ ID NO: 2) of WIPK and affinity purified. The specificity of the Ab-p44N was assessed by immunoblot analysis against a panel of different MAP kinases as described above for Ab-p48N. Ab-p44N recognized only the His-tagged WIPK protein (Fig. 2A). Addition to the immuno reaction of the competitor peptide p44N, but not the p48N, blocked binding of Ab-p44N to the His-tagged WIPK protein

(Fig. 2A), further demonstrating the specificity of this antibody.

(Page 36, line 13) WIPK was originally isolated based on an increase in its mRNA level after wounding; it was presumed to encode a wounding-activated 46-kD MAP kinase (Seo et al., 1995). We have confirmed that wounding transiently induces *WIPK* at the mRNA level. However, there is little or no increase in WIPK protein following this very transient induction of *WIPK* mRNA. Furthermore, using the WIPK- and SIPK-specific antibodies Ab-p44N and Ab-p48N, respectively, we have discovered that the wounding-activated kinase is the 48-kD SIPK, not the 44-kD WIPK (data for SIPK set forth in Zhang & Klessig, 1998). In this regard it should be noted that the molecular weight of the wounding-activated MBP kinase described by Seo et al. (1995) (46-kD) and by the present inventors [(48-kD)] (44-kD) is not significantly different. Such slight differences in estimated molecular weight based on SDS-PAGE occurs commonly among different laboratories.

Claim amendments

1. (Amended) A transgenic plant expressing an N gene, having enhanced resistance to a plant disease-causing agent selected from the group consisting of tobamoviruses, elicitin-producing fungi, parasiticein-producing fungi, cyrptogein-producing fungi, harpin-producing bacteria [and nematodes] tobacco mosaic virus and Phytophthora fungi; [said cell being] wherein said transgenic plant is stably transformed with a [DNA] nucleic acid construct comprising the figwort mosaic virus 35S promoter operably linked to a nucleic acid molecule selected from the group consisting of a sequence set forth in GenBank Accession No. D61377 or a sequence having 90% sequence identity therewith encoding a functional WIPK enzyme, said nucleic acid molecule being expressible in a plant cell[,

encoding WIPK].

10. (Amended) A method of making a transgenic plant expressing the N gene, having [with] enhanced disease resistance comprising:

a) transforming regenerable cells of a plant with a recombinant DNA construct comprising a figwort mosaic virus 35S promoter operably linked to a nucleic acid molecule selected from the group consisting of a sequence set forth in GenBank Accession No. D61377 or a sequence having 90% sequence identity therewith encoding a functional WIPK enzyme, expressible in a plant[, encoding a WIPK enzyme]; and

b) regenerating a transgenic plant from said transformed regenerable cells, said transgenic plant having enhanced disease resistance to a plant disease-causing agent selected from the group consisting of, tobamoviruses, elicitin-producing fungi, parasiticein-producing fungi, cyrptogein-producing fungi, harpin-producing bacteria, tobacco mosaic virus and Phytophthora fungi.

11. (Amended) The method of claim 10, wherein the DNA construct constitutively produces [the] a WIPK [protein] enzyme.

12. (Amended) The method of claim 10, wherein the DNA construct inducibly produces [the] a WIPK [protein] enzyme.

15. (Amended) The method of claim [14] 10, [which produces a plant having] wherein said transgenic plant is tobacco and has enhanced resistance to tobacco mosaic virus.

16. (Amended) The method of claim [14] 10, [which produces a plant having] wherein said transgenic tobacco plant has enhanced resistance to species of the fungal genus *Phytophthora*.